Mobilization of Mercury and Arsenic in Humans by Sodium 2,3-Dimercapto-1-propane Sulfonate (DMPS)

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Sodium 2,3-dimercapto-1-propane sulfonate (DMPS, Dimaval) is a water-soluble chelating agent that can be given by mouth or systemically and has been used to treat metal intoxication since the 1960s in the former Soviet Union and since 1978 in Germany. To better approximate the body burdens of Hg and As in humans, DMPS–Hg and DMPS–As challenge tests have been developed. The tests involve collecting an overnight urine, administering 300 mg DMPS at zero time, collecting the urine from 0 to 6 hr, and determining the urinary Hg before and after DMPS is given. The challenge test, when applied to normal college student volunteers with and without amalgam restorations in their mouths, indicated that two-thirds of the Hg excreted in the urine after DMPS administration originated in their dental amalgams. In addition, there was a positive linear correlation between the amalgam score (a measure of amalgam surface) and urinary Hg after the challenge test. When the DMPS–Hg challenge test was used to study dental personnel occupationally exposed to Hg, the urinary excretion of Hg was 88, 49, and 35 times greater after DMPS administration than before administration in 10 dental technicians, 5 dentists, and 13 nondental personnel, respectively. DMPS also was used to measure the body burden of humans with a history of drinking water containing 600 μg As/liter. DMPS administration resulted in a tripling of the monomethylarsonic acid percentage and a halving of the dimethylarsinic acid percentage as related to total urinary As. Because South American animals studied were deficient in arsenite methytransferase, a hypothesis is presented that arsenite and arsenite methyltransferase may have had a role in the evolution of some South American animals. — *Environ Health Perspect* 106(Suppl 4):1017–1025 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-4/1017-1025aposhian/abstract.html*

Key words: DMPS, arsenic, mercury, chelation, Chile, challenge test

Concerns have increased about neurotoxicants such as methylmercury in fish (*1*), elemental Hg vapor emitted by dental amalgams (*2–5*), lead in paint (*6*), and inorganic arsenic (inorgAs) in the drinking water of the United States (*7*), Chile (*8–10*), Mexico (*11*), Argentina (*12*), India (*13,14*), China (*15*), and Taiwan (*16*). Because continued efforts to maintain clean living and working environments are necessary, the use of antidotes and prophylactics (Table 1) for these toxic heavy metals and metalloids are sometimes minimized because of the fear that their use might decrease efforts to maintain and ensure a healthy environment.

Our laboratory had a role in the development of two of the relatively new chelating agents, DMPS [sodium 2,3-dimercapto-1-propane sulfonate (Dimaval); Heyl, Berlin, Germany] and DMSA (*meso*-2,3-dimercaptosuccinic acid, succimer). We developed analytical procedures for their study (*18,19*), investigated their metabolism (*20–25*), and honed their therapeutic uses (*3,9,26,27*). The purpose of this paper is to review our studies of the use of DMPS as a challenge or provocative test for Hg (*3,26,27*) and As in humans (*9*). A number of reviews dealing with DMPS and DMSA are available (*17,28–32*).

Although BAL (British Anti-Lewisite, dimercaprol, 2,3-dimercapto-1-propanol) has been the drug of choice for the treatment of As, lead, and even Hg intoxication in the United States since the late 1940s, it has many disadvantages (*33*). Approximately 55% of the patients receiving BAL have some kind of unpleasant side effect (*33*). This and the fear of the As-containing chemical warfare agent Lewisite resulted in the development of two water-soluble, orally useful chemical analogs of the lipid-soluble BAL.

DMPS was developed in the 1950s in the former Soviet Union by Petrunkin (*34*) and became an official drug in the Soviet physician's armamentarium in 1958 (*35*). Because it had potential use as an antidote for the chemical warfare agent Lewisite, it was not available outside of the Soviet Union until 1978, at which time Heyl, a small pharmaceutical company in Berlin, Germany, specializing in antidotes, announced its synthesis and distribution. Since then, it has been widely used as a chelating agent for both diagnostic and treatment purposes, especially in Germany, where for many years a physician's prescription was not required for its purchase (*36*). Its major use has been for mobilizing inorganic Hg in the body [reviewed by Kemper et al. (*17*), Aaseth et al. (*28*), Aposhian et al. (*29*), and Aposhian (*30*)] It has been used largely because of the increasing concerns about elemental Hg emission from dental amalgams *in vivo* (*2–5*) and about dental personnel exposed occupationally to Hg (*26,37*). DMPS is approved for use in Germany. It is still an investigational drug in the United States where an investigational new drug permit (IND) is required for its research use in humans. A compassionate IND can be obtained quickly from the U.S. Food and Drug Administration for DMPS use in emergencies such as life-threatening situations.

DMPS capsules (Dimaval) were gifts of Heyl (Berlin, Germany). It is appropriate to note that in Europe DMPS is manufactured and distributed by three different companies. Only Heyl's Dimaval

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Abbreviations used: BAL, British Anti-Lewisite; dimercaprol, 2,3-dimercapto-1-propanol; CDC, Centers for Disease Control and Prevention; DMA, dimethylarsinic acid; DMPS, sodium 2,3-dimercapto-1-propane sulfonate; DMSA, *meso*-2,3-dimercaptosuccinic acid, succimer; IND, investigational new drug permit; inorgAs, inorganic arsenic; MMA, monomethylarsonic acid; TotAs, total arsenic = inorgAs + MMA + DMA.

Table 1. Indications and contraindications of chelating agents in heavy metal poisonings (provisional recommendations).

Metal	First choice	Second choice	Contraindication
Hg metal	DMPS	DMSA	Dimercaprol
Hg inorganic	DMPS	DMSA	Dimercaprol
Hg organic	DMSA	DMPS	Dimercaprol
Pb	DMSA	DMPS	Dimercaprol, EDTA (?)
As	DMPS, DMSA	Dimercaprol	Dimercaprol (?)
Cr	DMPS	—	—
Sb	DMPS	—	—
Transuranics	DTPA	—	—

Data from Kemper et al. (*17*).

is manufactured by approved Western pharmaceutical procedures.

Since the development in the 1940s of chelating agents for therapeutic use in metal and metalloid poisonings, DMPS and DMSA have been the most selective and specific. Of these two orally useful chelating agents, DMPS has at least three advantages. First, it appears to remain in the body for a longer time than DMSA (*25*). Second, it acts more quickly than DMSA, probably because its distribution is both extracellular and intracellular (*38,39*). DMSA, however, appears to be only extracellular in its distribution (*39*). Third, preparations of DMPS are available for intravenous or intramuscular use. An intravenous preparation is a distinct advantage when metal toxicity has been so severe that the poisoned patient must be put on dialysis or when immediate use in the emergency room is necessary. DMSA, however, is available only in capsule form. In addition, the original licensing of DMSA by Johnson & Johnson Baby Products (Skillman, NJ) and to McNeil (Fort Washington, PA) and eventually to two smaller pharmaceutical companies has made its availability questionable, to say the least. For example, DMSA appears to be unavailable for human research or therapeutic use in Europe at this time. The major research interest in DMSA at present appears to be a large-scale clinical trial in the United States to ascertain whether it can reverse some of the cognitive damage produced by chronic lead exposure in U.S. children. Unfortunately, the trial appears to be moving slowly because of low enrollment and compliance.

A number of years ago we began to evaluate the use of DMPS as a challenge test for Hg. We used the oral capsules (Dimaval) in most of our studies (*3,26,27*). Other investigators have used the injectable preparations (Dimaval) for challenge tests (*40,41*). However, we used the parenteral preparation for our intravenous pharmacokinetic studies (*25*). We prefer using DMPS capsules because of the ease of administration and because side effects to most drugs are less likely to occur when they are given orally.

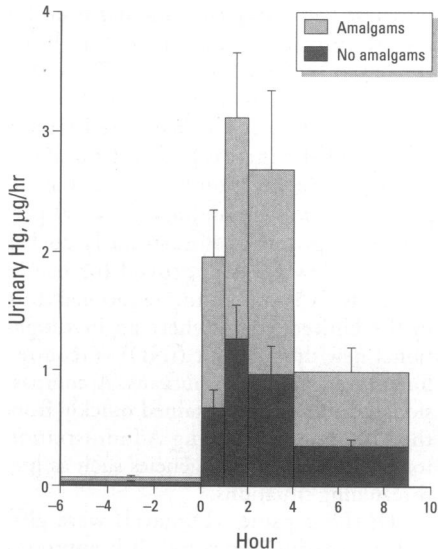


Figure 1. Urinary Hg before and after administration of 300 mg DMPS to volunteers with and without dental amalgams.

Increased Urinary Excretion of Mercury

Normal Humans

There has been considerable controversy whether the elemental Hg emitted from dental amalgams in humans causes adverse health effects and whether this Hg adds to the body burden of this extremely toxic heavy metal (*2,42*). Most dental amalgams contain as much as 50% metallic Hg. To determine whether dental amalgams influence the body burden of Hg, college students (15 males and 5 females) with and without dental amalgams were chosen as subjects. The diameters of each of the surfaces of all the dental amalgam restorations in each subject's mouth were measured, a score determined for each surface, and the scores summed to obtain the amalgam score (*3*). After an overnight fast, administration of three 100-mg DMPS capsules by mouth increased the mean urinary Hg excretion of the amalgam group and the nonamalgam group over a 9-hr period (Figure 1; Table 2). Our first conclusion from these experiments (*2*) was that DMPS can be used to increase the urinary excretion of Hg, a confirmation of many studies by others (*43,44*). Our second conclusion was that two-thirds of the Hg excreted in the urine of subjects with dental amalgams appeared to be derived from the Hg vapor released from their amalgams (Figure 1). Linear regression analysis indicated a highly significant positive correlation between the Hg excreted in the urine 2 hr after DMPS administration and the dental amalgam scores (Figure 2). The third conclusion was that because the urinary Hg concentration of normal individuals is barely detectable by the cold vapor atomic absorption analytical procedure we used, the significance and reliability of these measurements can be increased by determining urinary Hg after a DMPS challenge.

Dental Technicians, Dentists, and Nondental Personnel

We were asked to evaluate the Hg body load of dental personnel working in a new facility in a developing country. For economic reasons, the dental technicians in this clinic formulate the dental amalgam as needed by taking a few drops of Hg from a bottle and putting them on a piece of filter paper. They add to this some amalgam alloy and carry it to the dentist, who

Table 2. Urinary mercury excretion before and after the oral administration of 300 mg DMPS to normal individuals with and without dental mercury amalgams.

	Group, µg Hg ± SE[a]		
	No amalgam	Amalgam	*p*
−9 to 0 hr	0.27 ± 0.04	0.70 ± 0.11	< 0.002
0 to 9 hr	5.10 ± 1.11	17.16 ± 3.32	< 0.003
p	< 0.001	< 0.001	–

[a] $n = 10$ for each group. DMPS was given at zero time.

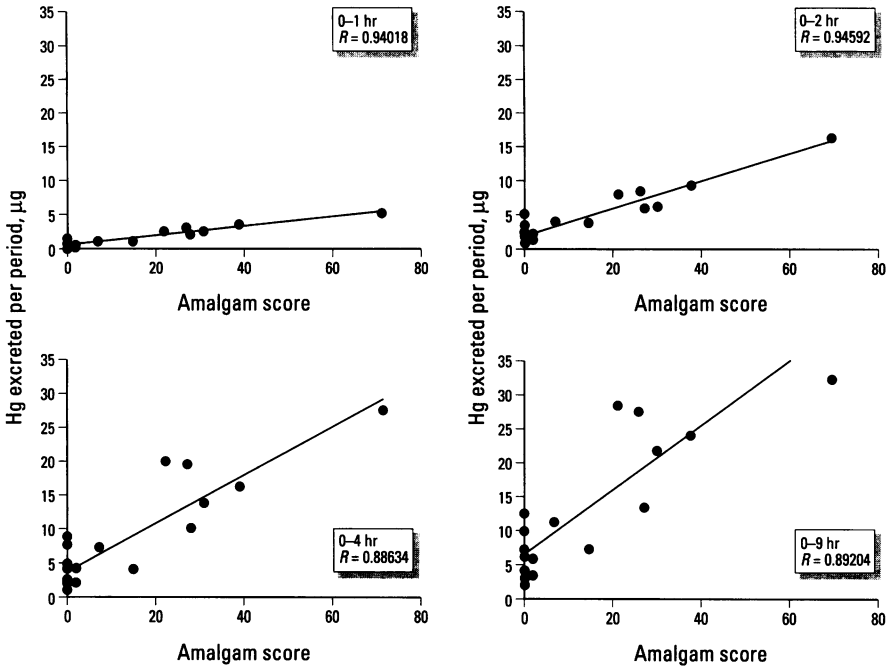


Figure 2. Amalgam score versus urinary Hg excretion after DMPS administration. The Hg excretion after DMPS administration has been corrected by subtracting the Hg excretion found for the same length of time prior to administering DMPS. This was done by collecting the urine for 11 hr before DMPS administration and determining the mercury content per 11 hr, then calculating the mercury excretion per hour. *R*, coefficient of correlation.

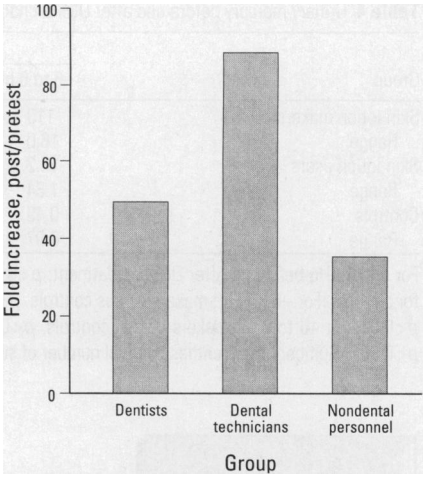


Figure 3. Increase of urinary mercury excretion after Dimaval challenge test of dental technicians, dentists, and controls in Monterrey, Mexico.

Table 3. DMPS challenge test for mercury.

Time	Action
–11 to 0 hr	Begin fast Begin overnight urine collection
0 hr	End overnight urine collection No breakfast, no coffee, no tea Administer three 100-mg DMPS capsules Begin 0 to 6 hr urine collection
+4 hr	Eat chicken or turkey sandwich
+6 hr	Empty bladder, end both urine collection and the fast Acidify urine and freeze until analyzed Analyze for total Hg by cold vapor atomic absorption

squeezes out the excess Hg. Because of our findings (*26*) as to the amount of Hg excreted by the dental technicians after DMPS was given (Figure 3), the clinic has begun to use amalgam capsules. In these capsules, the Hg and alloy powder are separated by a partition, which is broken by shaking the capsule vigorously. Thus, the amalgam is formulated with less Hg exposure to dental personnel.

The DMPS challenge test (300 mg by mouth after an 11-hr fast; Table 3) was given in Monterrey, Mexico, to 10 dental technicians (all females), 5 dentists (4 males and 1 female), and 13 nondental personnel (8 males and 5 females) to ascertain their occupational exposure to Hg used in the preparation of amalgams (*26*). Urines were collected and analyzed for total Hg. Mean Hg urinary excretion 6 hr before and 6 hr after DMPS administration for the dental technicians (who formulate amalgam) was 4.84 µg ± 0.742 standard error (SE) and 424. µg ± 84.9 SE; for the dentists (who use amalgam in their practice) 3.28 µg ± 1.11 SE and 162.0 µg ± 51.2 SE; and for the nondental personnel 0.783 µg ± 0.189 SE and 27.3 µg ± 3.19 SE. (These control values appear to be different from the nondental personnel values in the "Normal Humans" section. This may be due to economic and dietary differences because this group consisted of laborers and the previous group consisted of research laboratory and medical personnel.) The increase in urinary Hg excretion before and after DMPS administration was considerable (Figure 3). The urinary coproporphyrin levels before DMPS administration, indicative of renal Hg content, were quantitatively associated with the urinary Hg levels among the three study groups after DMPS administration (*26*). This was not so when the urinary Hg before DMPS administration was compared to urinary coproporphyrin. Thus it appears that the urinary Hg level after DMPS administration is a better indicator of exposure and renal Hg burden than the Hg measured in the urine before DMPS is given. Regression analysis showed that the coefficient of urinary Hg was statistically and adversely associated with complex attention (switching task), the perceptual motor task (symbol–digit substitution), symptoms, and mood. The easily performed DMPS–Hg challenge test is useful for monitoring humans for Hg vapor exposure (*26*).

Factory Workers, Skin Lotion Users, and Controls

We previously administered the DMPS challenge test to humans exposed to the elemental Hg (vapor) of amalgams (*3,26*), but not to mercurous salts. The challenge test was given to 11 factory workers who made a skin lotion containing mercurous chloride, 8 users of the skin lotion, and 9 controls (*27*). Urines were analyzed for total Hg by using cold vapor atomic absorption spectrophotometry. The Hg excreted for 6 hr before and 6 hr after DMPS administration was 113 µg ± 26 SE and 5037 µg ± 682 SE for the skin lotion makers; 16.2 µg ± 3.4 SE and 1410 µg ± 346 SE for the skin lotion users; and 0.49 µg ± 0.11 SE and 18.4 µg ± 7.1 SE for the controls, respectively (Table 4). The increases in urinary Hg resulting from the DMPS–Hg challenge test were 45-, 87-, and 38-fold, respectively. The results demonstrate that in humans exposed to mercurous chloride, DMPS increased the urinary excretion of Hg and that the DMPS–Hg challenge test is of value for a more realistic estimation of mobilizable Hg in humans (*27*).

Table 4. Urinary mercury before and after DMPS challenge test.

Group	μg Hg ± SEM	
	–6 to 0 hr (before)	0 to +6 hr (after)
Skin lotion makers	113 ± 26 (11)	5037 ± 682 (11)
Range	16.0 – 314	1728 – 10,307
Skin lotion users	16.2 ± 3.4 (8)	1410 ± 346 (8)
Range	1.84 – 35.3	71.8 – 3075
Controls	0.49 ± 0.11 (8)	18.4 ± 7.1 (8)
Range	0.07 – 0.98	3.17 – 54.2

For urinary Hg before vs after DMPS treatment: $p<0.001$ for lotion makers; $p<0.002$ for lotion users; and $p<0.05$ for controls. For –6 to 0 hr: makers versus controls, $p<0.002$; users versus controls, $p<0.001$; makers versus users, $p<0.01$. For +6 to 0 hr: makers versus controls, $p<0.001$; users versus controls, $p<0.001$; makers versus users, $p<0.001$. Numbers in parentheses equal number of subjects included in the mean.

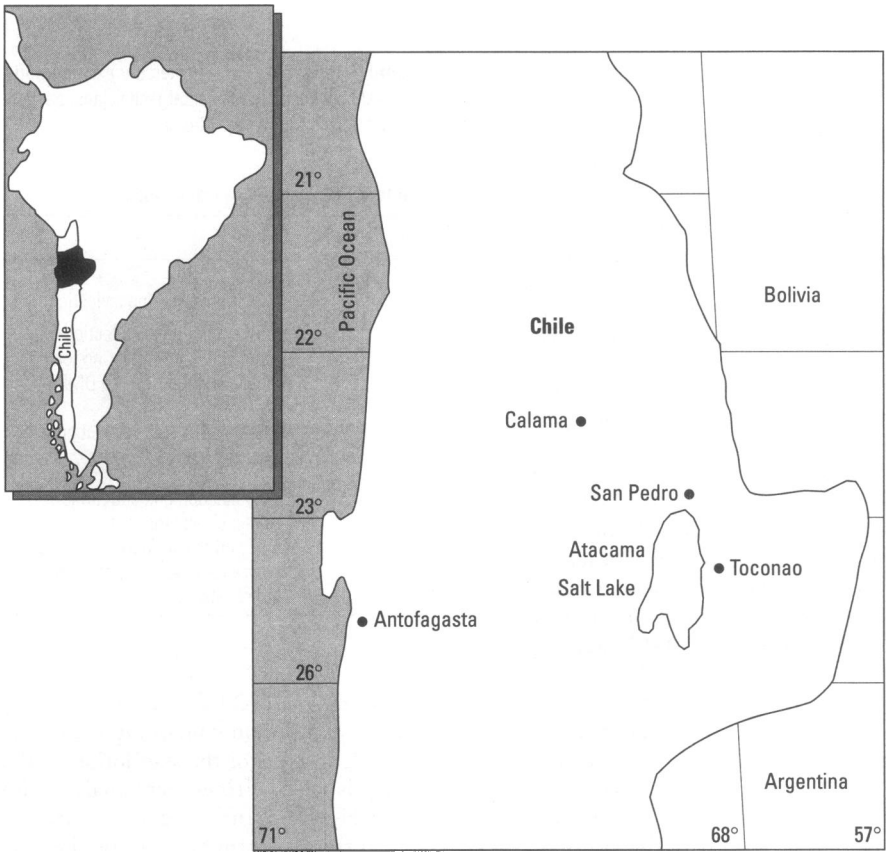


Figure 4. Map showing locations of San Pedro de Atacama and Toconoa in the Antofagasta Province of Chile. Data from Hopenhayn-Rich (*10*).

DMPS–Arsenic Challenge Test

In May 1995 we were given the opportunity to study how the DMPS challenge test might alter the urinary As in humans chronically exposed to As in their drinking water. San Pedro de Atacama in Chile was our study town. It is a relatively isolated town in the Atacama Desert in northeast Chile (Figure 4). The drinking water for San Pedro de Atacama is obtained from a river having an As concentration of 593 μg/l. The water has contained high levels of As for centuries. The source of the As is the runoff from high volcanic formations in the Andes. Toconao, the control town, is about a 1-hr drive beyond San Pedro de Atacama. Residents of Toconao drink water containing about 19 μg As/l. The protocol for the DMPS–As challenge test (Table 5) differs from the DMPS–Hg challenge test (Table 3) only by the urine collection schedule.

Table 5. DMPS–arsenic challenge protocol.[a]

Time	Action
–11 to 0 hr	Begin fast Begin overnight urine collection
0 hr	End overnight urine collection No breakfast, no coffee, no tea Administer three 100-mg DMPS capsules Begin 0–2 hr urine collection
2 hr	End 0–2 hr urine collection Begin 2–4 hr urine collection
4 hr	End 2–4 hr urine collection Begin 4–6 hr urine collection Eat chicken or turkey sandwich
6 hr	End 4–6 hr urine collection Begin 6–24 hr urine collection
11 hr	Eat dinner
24 hr	End 6–24 hr urine collection

[a]Physical examination and vital signs were measured before and after the study. All urines were acidified and frozen until analyzed for As species by hydride generation atomic absorption.

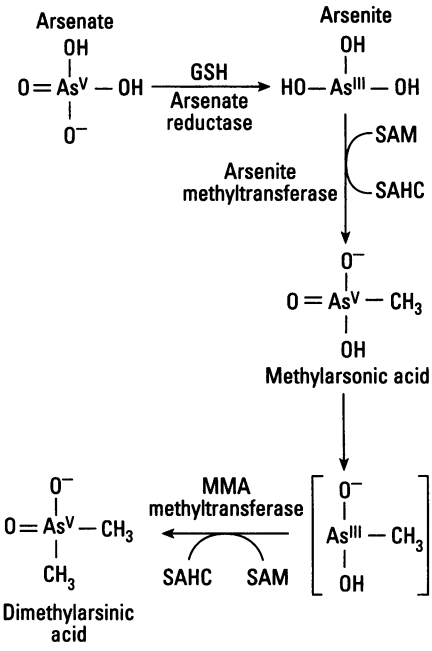


Figure 5. Putative pathway for biotransformation of arsenate/arsenite. GSH, glutathione; SACH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.

Amount of Arsenic Species in Urine

The amount of As species in urine is often considered an indication of chronic As exposure. The species are the result of the biotransformation of inorgAs (Figure 5). Although the concentrations of the various As species in the urine after the DMPS challenge were determined (*9*), the

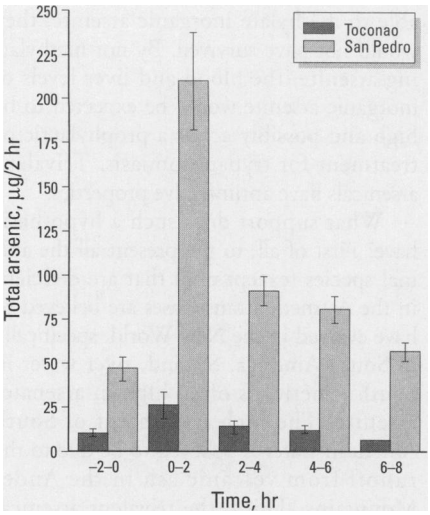


Figure 6. Total arsenic excreted in the urine before and after administration of 300 mg DMPS by mouth. DMPS was given at zero time. Although urine was collected overnight (–11 to 0 hr) and for 6–24 hr, the total amount of arsenic excreted for these times was divided by 5.5 and 9 to make the data comparable to the 2-hr urine collection periods of 0–2, 2–4, and 4–6 hr. Error bars ± SE.

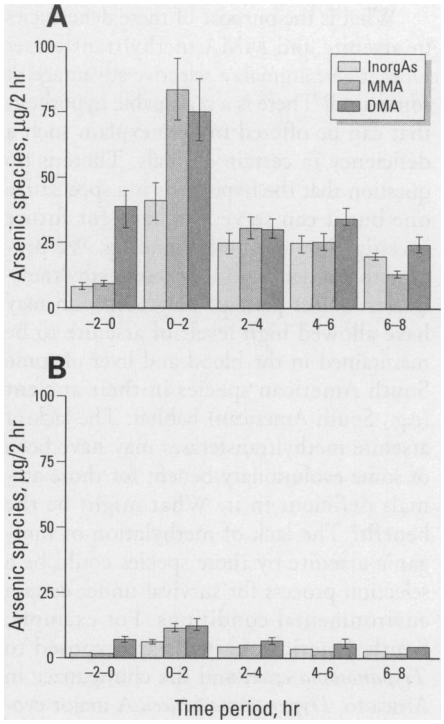


Figure 7. Urinary excretion of inorganic arsenic, MMA, and DMA during 2-hr time periods before and after administration of 300 mg DMPS orally. (*A*) San Pedro de Atacama subjects. (*B*) Toconao (control) subjects. DMPS was given at zero time. Although urine was collected overnight (–11 to 0 hr) and for 6–24 hr, the total amount of As excreted for these times was divided by 5.5 and 9, respectively, to make the data comparable to the 2-hr urine collection periods of 0–2, 2–4, and 4–6 hr. Error bars ± SE.

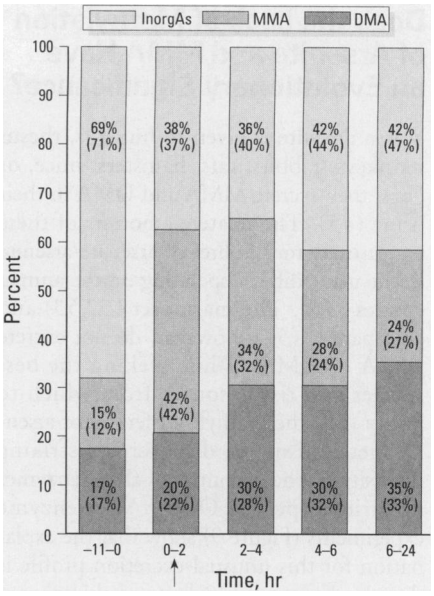


Figure 8. Arsenic species expressed as a percentage of urinary total arsenic at time periods before and after DMPS administration. DMPS (300 mg) was given by mouth at zero time. Percentages for the Toconao group are in parentheses. Arrow denotes DMPS given at 0 time.

amounts of these species were of greater interest because they are a better indicator of the body burden of As. As compared to the period before DMPS administration, the mean total As (TotAs) in the urine of the San Pedro de Atacama subjects increased approximately 4-fold during the 2-hour period after DMPS (Figure 6). When the San Pedro de Atacama and Toconao subjects were compared, there was a striking difference noted between the mean amount of TotAs excreted in the urine during all time periods (Figure 6). This was not surprising because residents of the two villages drink water containing vastly different As concentrations (*9*).

It was surprising that there was a marked increase in the amount of urinary monomethylarsonic acid (MMA) being excreted in the urine of both the San Pedro and Toconao groups after DMPS was given (Figure 7). There was approximately a 9-fold increase in urinary MMA during the 2-hr period after DMPS administration as compared to the previous 2 hr. For the same time periods, urinary inorgAs was increased about 5-fold and dimethylarsinic acid (DMA) less than 2-fold. From 2 to 6 hr after DMPS, the amount of inorgAs, MMA, and DMA did not change to any great extent. For the Toconao subjects, although the absolute amounts of As species per 2-hr period were much less, the fold increases relative to the preceding time period were similar.

Percent of Arsenic Species in the Urine

The percentage that a urinary As species is of the total urinary As has been used as a criterion of the normal biotransformation or metabolism of As in a given species and in a given individual (*45*). The most striking change in our Chilean study was that the percent MMA increased to an extent never seen in any previously reported study in humans or animals (Figure 8). The mean increased from 15% (San Pedro de Atacama) and 12% (Toconao) before DMPS administration to 42% for each group during the 0- to 2-hr period after DMPS. The usual percentage of MMA reported in human urine has had a range of 10 to 20% (*46–48*). The increase in percent MMA appeared to be accompanied by a substantial decrease in percent DMA. The changes in the percent of urinary As species for the Toconao group were very similar to those found for the San Pedro de Atacama group (Figure 8).

The percent inorgAs in the urine increased after DMPS administration (Figure 8). By the end of 6 hr it had decreased to 28 and 24% for San Pedro de Atacama and Toconao, respectively. For urinary DMA, however, the percentage decreased after DMPS. Although DMPS administration resulted in significant changes in the percent of these various As species excreted in the urine of both San Pedro de Atacama and Toconao subjects, the magnitude of the changes in relative percent was essentially the same for both groups (Figure 8).

Other Observations

There were no signs of As toxicity in the San Pedro de Atacama residents as far as skin keratosis and ulcerations. The lack of toxic signs and symptoms of chronic As toxicity is highly unusual. One cannot help but wonder whether the difference between the San Pedro subjects, where exposure has continued for at least 10,000 years, and the victims of As exposure in Taiwan, Mexico, and India, where exposure is relatively recent, is a matter of polymorphism or survival of the fittest. Along these lines, recent studies from our laboratory (*49–51*) indicate South American animals lack the enzymes responsible for the methylation of inorganic arsenite to the supposedly less toxic methylated As species.

Does the Lack of Methylation of Arsenite and MMA Have an Evolutionary Significance?

When arsenite is given to humans, rhesus monkeys, rabbits, rats, hamsters, mice, or dogs, they excrete MMA and DMA in their urine (*45*). The relative amounts of these two urinary metabolites of arsenate/arsenite in the urine differ depending on the animal species (*45*). The marmoset (*52,53*) and chimpanzee (*54*), however, do not excrete MMA or DMA. While seeking the best species and tissue source from which to purify (*55*) the methyltransferases of arsenite metabolism, we discovered a striking diversity in the amounts of these enzymes in various species. Our *in vitro* enzyme experiments (Figure 9) show that the explanation for this unusual excretion profile is that the marmoset monkey and chimpanzee are deficient in or lack active arsenite methyltransferase(s) (*49,56*). The marmoset is a New World monkey. An Old World monkey, the rhesus, has ample As methyltransferase activity. The chimpanzee is a subhuman primate from the Gold Coast of western Africa and Nigeria. Geographically, western and central Africa are between the rain forests of South America and the plains of India. The rhesus monkey seems to have evolved mainly from the India subcontinent. Our future plans are to try to narrow these geographic areas of arsenite methyltransferase diversity.

What is the purpose of these deficiencies in arsenite and MMA methyltransferase? Does it give animals a selective advantage of some kind? There is a reasonable hypothesis that can be offered to help explain such a deficiency in certain animals. There is no question that the hypothesis is a speculative one but it can serve as a basis for future investigation and understanding. We propose that a deficiency of these methyltransferases and/or perhaps polymorphism may have allowed high levels of arsenite to be maintained in the blood and liver of some South American species in their ancient (e.g., South American) habitat. The lack of arsenite methyltransferases may have been of some evolutionary benefit for those animals deficient in it. What might be the benefit? The lack of methylation of inorganic arsenite by these species could be a selection process for survival under certain environmental conditions. For example, South American animals are exposed to *Trypanosoma cruzi* and the chimpanzee in Africa to *Trypanosoma brucei.* A major evolutionary demand is the preservation of the species. The lack of the arsenite methylating enzymes in the marmoset (*49*), tamarin (*49*), and squirrel monkeys (*56*), guinea pig (*50*), and chimpanzee (*56*) may be the result of evolutionary selection necessary for survival of the species in an environment containing lethal trypanosomes or other pathogens in South America and Africa. We propose that if these animals had been able to methylate inorganic arsenite, they would not have survived. By not methylating arsenite, the blood and liver levels of inorganic arsenite would be expected to be high and possibly act as a prophylactic or treatment for trypanosomiasis. Trivalent arsenicals have antiinfective properties.

What support does such a hypothesis have? First of all, to the present all the animal species (except one) that are deficient in the As methyltransferases are believed to have evolved in the New World, specifically in South America. Second, river water in South America is often high in arsenate/arsenite. The high As content of South American water is believed to be due to the runoff from volcanic ash in the Andes Mountains. Third, the trivalent arsenical melarsen oxide has been the drug of choice, until recently, to treat late-stage African trypanosomiasis *Trypanosoma brucei gambiense* (sleeping sickness). Its mechanism of action is thought to be the formation of an adduct with reduced trypanothione, a reduced glutathionelike compound. Trypanothione is N^1N^8-bis(glutathionyl)spermidine (Figure 10). It is the major intracellular thiol of *T. brucei* and other trypanosomes (*57*). In fact, it makes up more than 68% of the intracellular thiol of these organisms (*58*). Melarsen oxide forms an adduct with reduced trypanothione (Figure 10). The adduct inhibits trypanothione disulfide

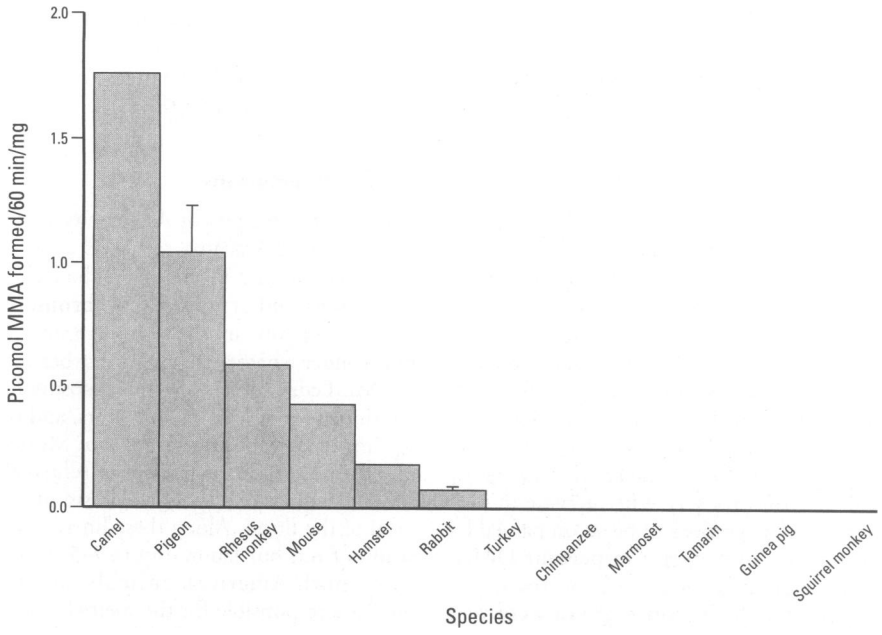
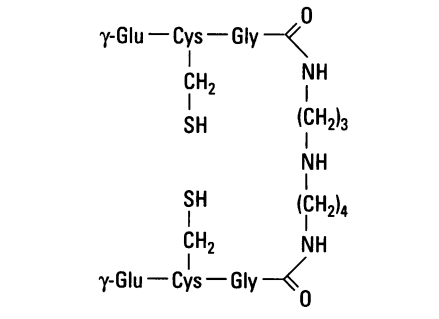


Figure 9. Arsenite methyltransferases of liver cytosols of various animals.



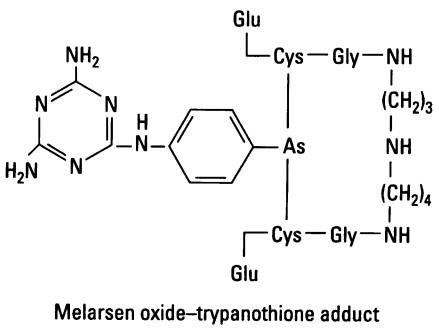


Figure 10. Trypanothione and its melarsen oxide adduct.

reductase, an enzyme unique and essential to trypanosomatids and leishmania, resulting in a decrease of intracellular reduced trypanothione. Fourth, Chagas' disease, caused by *Trypanosoma cruzi*, is endemic in South America. Fifth, up to now, the exception to our hypothesis about only New World animals being deficient or lacking arsenite and MMA methyltransferases is the chimpanzee, which appears to have evolved in the Old World. The chimpanzee's natural home, however, is the northwestern and central area of Africa—an area that once was bound to or had island bridges to South America. In this area of Africa, *T. brucei gambiense* is endemic. It is not unreasonable, then, to consider that at one time the environment of the marmoset, guinea pig, and chimpanzee may have had something in common. Seventh, the pigeon, rat, hamster, rhesus monkey, and mouse have arsenite methyltransferase activity and are considered Old World as far as their evolution is concerned.

Buchet's group (*59,60*) and Thomas' group (*61,62*) also studied the methyltransferases of As metabolism in rat liver homogenates. We have now purified the rabbit arsenite methyltransferase 4200-fold and it is being analyzed for its amino acid sequences. Once the sequences are known, nucleotide probes can be synthesized that may be useful in determining the occurrence of these enzyme activities in lymphocytes and other human material readily available for biomarker studies, especially in humans exposed to inorganic As in the water they drink, the food they eat, and/or the air they breathe.

Discussion

The DMPS–Mercury Challenge Test

One might wonder why we think there is a need for such a challenge test. Let us for a moment consider the recent history of lead. In 1970, the Centers for Disease Control (CDC) considered a lead level of 40 µg/dl blood to be of medical concern (*6*). In sharp steps (Figure 11), this has been reduced to 10 µg/dl over a 17-year period. There are many toxicologists, however, who believe that there is no safe level of lead. A DMPS–Hg challenge test may help obtain specific information so that the calamity caused by lead among many young children in the United States will not be repeated with Hg. At possible risk from Hg are young children, women who obtain dental care while pregnant, and their potentially exposed fetuses. Whether the amount of Hg emitted from dental amalgams can cause harmful human health effects is at present uncertain; the safety of dental amalgams has not been proven. One has only to look at Figure 11, showing how the blood lead concentrations of concern have changed over the last 20 years, to realize that such changes might also happen to urinary Hg levels of concern. Investigators studying toxicology and human health must determine whether dental amalgam Hg is harmful or not.

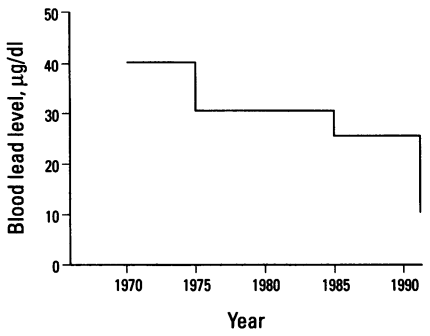


Figure 11. Blood lead levels considered elevated by the CDC and the Public Health Service.

Since our DMPS studies of users and makers of a mercurous chloride-containing skin-lightening lotion (Table 3) were completed, a miniepidemic involving Hispanic women in the southwestern states of Texas, New Mexico, Arizona, and California has been declared by the CDC (*63*). Even more surprising have been the elevated urinary Hg levels of some of their young children. According to the mothers' statements, the lotion was not applied to the children. Although resources were mobilized to remove this lotion from Hispanic stores in the United States, unfortunately no federal funds were made available for physicians to follow the health of these exposed women of childbearing age and who belong to an important minority group in our country.

Our studies with DMPS show clearly that urinary Hg after a DMPS challenge test is a better indication of the body burden of Hg in a human. If the body is compared to a coffee cup, the usual urinary Hg concentration may be compared to the overflow of that cup. Using DMPS would be comparable to tipping the cup so that the fluid pouring out would be more indicative of what previously remained in the cup (body). Clinicians and research investigators often consider urine differently. Many physicians consider heavy metal concentrations in the urine an excellent diagnostic tool. Researchers, however, want to know about what was left behind in the body, thus doing the damage. This is why the DMPS challenge information is of value. It uses the urine after a DMPS challenge to tell us more about what previously remained in the body.

DMPS and Arsenic

The results with DMPS and As are of great interest because the unique increase in urinary excretion of MMA after DMPS administration is very important. The chemical structure of the DMPS–As chelate being excreted is not known. The form of As necessary for chelation must be As^{III}. Could it be that the chelate is the elusive MMA species containing As^{III}, or is it arsenite? We do not know the answer to this question as yet but we are investigating. Other thoughts about the possible mechanisms and significance of this increase in the urinary MMA percent can be found in Aposhian et al. (*9*).

Finally, we wish to emphasize that these arsenite methyltransferase enzymes have species diversity as well as species polymorphism, and thus have potential as important biomarkers for As exposure. In addition, the lack of the arsenite-methylating enzymes in marmoset, tamarin, and squirrel monkeys, guinea pig, and chimpanzee may be related to evolutionary selection necessary for survival in a hostile environment containing death-causing parasites of some kind. The animals that could methylate inorganic As species did not survive. Those that could not methylate did survive. This is our working hypothesis that is now under investigation.

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